QUANTIFICATION OF VERTICAL-FIBER DEFECT IN CATTLE HIDE BY SMALL-ANGLE LIGHT SCATTERING

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Vertical-fiber defect (VFD), an abnormal arrangement of collagen fibers in hides of certain cattle breeds, is still not fully understood. Prior work has been limited to subjective histological examinations from hide biopsies. A device using small angle light scattering (SALS) was used to quantify the collagen fiber orientation of sections taken from hide biopsies. Sections were chosen from the Hereford cattle breed and classified by conventional observation as belonging to either the normal, intermediate, or vertical phenotypes. The vertical fibers occur only in the upper reticular dermis, with the fibers in the lower reticular dermis lying parallel to the plane of the hide in all phenotypes. By SALS the vertical phenotype was found to be significantly different from the normal phenotype, whilst the intermediate phenotype was found to be structurally indistinguishable from the vertical one. No evidence was found for the existence of other phenotypes.

KEYWORDS: light scattering, vertical-fiber defect, bovine skin

INTRODUCTION

Vertical Fiber Defect (VFD) is a structural anomaly of collagen fibers in the upper reticular dermis of cattle hides. Since it was first reported by Amos,[1] many papers have been written in attempts to explore its nature.[2–7] The trait is characterized by a preferred orientation of the collagen fibers in the direction perpendicular to the plane of the hide (Fig. 1), especially in the thickest hide of the animal, in the upper rear quarter. It can involve up to 75% of the area of the trimmed hide. The tensile strength of a hide having VFD can be as much as 50% weaker than normal hide,[8] thus making it unusable for the production of many leather products. More seriously, VFD is often not detected until after the costs of leather manufacture have been incurred. An attempt was made by Everett[9] to use a preproduction mechanical evaluation to predict the occurrence of VFD, but was not successful. Thus, there is an economic as well as scientific interest in determining the exact nature of the trait.

The genotype has been found to occur only in the Hereford cattle line, involving a

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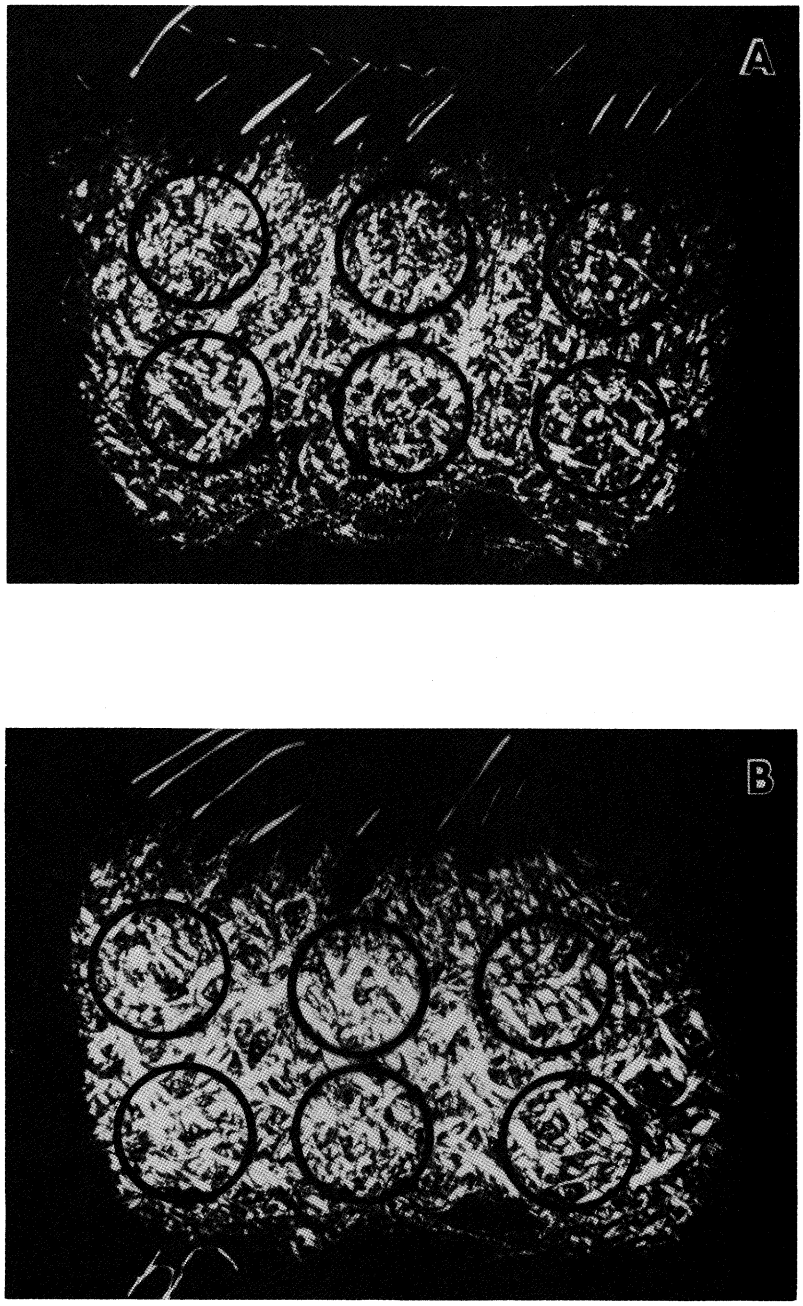

FIGURE 1 Hide sections used for SALS. Photomicrographs were taken in dark field with polarizers crossed perpendicular and parallel to the hide surfaces, brightening the fibers oriented at 45°. A: Vertical type. Perpendicular fibers are prominent in this section even though the 45° fibers are preferentially illuminated. B: Normal type. 45° fibers are prominent, selected by the polarization. Circles show approximately the six areas that were sampled by the laser beam in the reticular dermis, with hair follicles well out of the sampled field.

controlling autosomal recessive gene.[2,3] Although more than one gene may be involved in the degree of expression, the genetic evidence strongly suggests that one major gene determines this phenotype. This argument has been expanded in reference 2. A single genetic locus for vertical fibers would have important implications for skin morphogenesis in embryonic development. (An extreme possibility would be a single protein that controls fiber orientation in skin.)

In normal Hereford hide, the collagen fibers form compact bundles that interweave at an angle of about 40° to 60°, measured from the plane of the hide.[2,3,10] Fibers deeper in the dermis tend to be oriented more in the plane of the hide. In VFD the fibers are oriented vertically in the upper reticular dermis. Hannigan *et al.*[3] have classified the mutation into three phenotypes: (1) normal, i.e. normal hide, (2) intermediate, where the fibers are loosely interwoven with a variably upright (vertical) angle of weave, and appear vertical in localized areas, and (3) vertical, where the fibers are mostly vertical with little or no interweaving, as well as variants having an overall loose appearance. The terms "intermediate" and "vertical" are thought to represent two degrees of the VFD. Fibers in the lower reticular dermis tend to be oriented in the plane of the hide, and are unaffected by the occurrence of VFD in the upper reticular dermis.[1,10]

However, this description might not be as complete as once thought. In a paper by Peters and Bavinton,[10] it was suggested that the vertical and intermediate phenotypes are actually two different structural variants. The vertical phenotype was described as above, quite distinct from other phenotypes, as also noted by Amos.[1] The intermediate type, however, was proposed to be an extreme form of the normal phenotype with very high fiber angles (i.e., tending toward the vertical direction). Peters and Bavinton have called this structure "high-weave," and mentioned its occurrence in breeds other than Hereford. Unlike the vertical and intermediate phenotypes as defined by Hannigan *et al.*,[3] the high-weave condition extends throughout the thickness of the hide, with little interweaving of fibers. Further, high-weave may be the high end of a spectrum of weave angles naturally occurring in hide. Thus, the exact nature of VFD is still not clear.

The primary difficulty in the above classifications is that they rely on subjective observations of the collagen fiber architecture. Peters and Bavinton[10] have mentioned "that the separation of hides into normal and high-weave categories is a difficult and subjective decision." Thus, the whole analysis of the occurrence of intermediate forms of VFD is called into question. Clearly required is the development of a method to quantify the collagen fiber orientation. This is especially important in attempting to determine whether intermediate forms of VFD exist. There are various techniques to determine the orientation of collagen fiber networks, including X-ray diffraction.[14] The optical retardation of bovine reticular dermis cannot be uniformly compensated for determination of hirefringence because of the coarse fibrous structure. Kronick and Buechler[16] applied a small-angle light scattering (SALS) method to determine the fiber orientation distribution in calf hide. They compared their results to those from a parallel study using X-ray diffraction, and obtained close agreement. Thus, collagen fiber orientation could be successfully determined with SALS. In the present work, an automated SALS device was used to explore the collagen fiber orientation of histological sections from an earlier VFD study.[3] The main aim was to clarify the disparities found in the subjective evaluations of VFD. Structural information obtained from the SALS scattering patterns was evaluated. The results were compared to determine what differences exist among the normal, intermediate, and vertical phenotypes.

EXPERIMENTAL PROCEDURE

Histology

The biopsy samples used in this study had been evaluated histologically in a joint project between the Eastern Regional Research Center (ERRC) and the U.S. Meat Animal Research Center (USMARC). This project studied the occurrence and heritability of VFD.[3] The purebred Hereford cattle of known genealogy were located at USMARC. A biopsy sample 1 cm in diameter was taken from the rump area of each animal 25 cm from the tail and 25 cm lateral from the backbone, using an automatic biopsy gun.[11] The biopsies were taken from selected animals from December 1980 to September 1981. Each biopsy sample was put into 10% neutral formalin (a 1:10 dilution of 37% formaldehyde saturated with calcium carbonate) immediately after removal, and sent to the ERRC. All previous studies of VFD[1–7] had been conducted on samples fixed with formalin or commercial leather tanning agents. We have examined 60-μm frozen sections of fresh hide also and find the vertical layer to appear the same, at least for diagnostic purposes, as those fixed with either formalin or glutaraldehyde. The purpose of this study was therefore served by performing the usual histological diagnoses of VFD and the correlated SALS measurements on the same specimens.

The formalin-fixed biopsies were cut in half, perpendicular to the epidermal layer, in a plane parallel to the hair shafts, then sliced on a freezing microtome into cross-sections 60 μm thick. Sections were stained with hematoxylin and eosin (Lillie-Mayer variant 12), and mounted on standard microscope slides (Fig. 1). They were examined microscopically to classify the phenotypes of the individual animals as either normal (N), intermediate (I), or vertical (V). These classifications were consistent with the known geneologies of the animals.[3]

For this study, representative slides, prepared from the biopsy samples, were chosen from each of the three phenotype classifications. The slides selected were from Table I.

Small Angle Light Scattering (SALS)

The SALS device[12] consisted of an 0.4 mW-640 nm HeNe laser mounted on a one meter long optical bench, along with a beam expander and spatial filter. A stepper-motor driven rotator was used to rotate a stepper-motor driven translator, which had a 2-mm diameter CdS photocell mounted on it. This assembly was mounted on the optical bench 50 cm from the sample, in the light field scattered from it. By positioning the translator at the desired distance and turning the rotator, the photocell could measure the scattered light intensity distribution around concentric circles centered on the optic axis. Data acquisition and movement of the rotator and translator were controlled by a microcomputer (Keithly Series 500 Data acquisition and control device, Keithly-DAS Corporation, Boston, MA). The resolution of the rotator was 100 steps/degree; that of the translator, 1000 steps/mm, thus allowing high-resolution positioning of the photocell.

We believe the above to be the best configuration of the SALS device. For example, we tried rotating the slide while maintaining the photocell at a desired radius with the linear translator. Refraction caused by the microscope slide then caused the SALS pattern to wander about the optic axis as the slide was being rotated, distorting the light intensity readings. To avoid this problem, the microscope slide containing the sample must be held fixed on a separate slide holder.

TABLE I

Characteristics of the samples

Phenotype	Number	Age (months)	Sex
Vertical	2	12	M
Vertical	2	24	F
Intermediate	1	7	M
Intermediate	3	24	F
Normal	4	24	F

In order to characterize the fiber orientation of all samples, including variations within each sample, the sampling layout shown in Figure 1 was used. Three SALS patterns were taken in the upper reticular dermis at positions A, B, and C, and three in the lower reticular dermis at D, E, and F. Data from each pattern were stored and analyzed separately.

Analysis of SALS Data

Our analysis of the scattered light intensity distribution followed that which has been traditionally used in X-ray diffraction analysis.[13] Similar approaches have been used by Bigi[14] and Hukins[15] using X-rays to determine the collagen fiber orientation in connective tissues. Using these methods, the degree of orientation existing in a fibrous network was determined by the following equation:

$$\langle \cos^2 \phi \rangle = \frac{\int_0^{\pi/2} \int_0^{\pi} F(\phi, \beta) \cos^2 \phi \, d\phi d\beta}{\int_0^{\pi/2} \int_0^{\pi} F(\phi, \beta) d\phi d\beta} \tag{1}$$

$F(\phi, \beta)$ is the three-dimensional fiber distribution function. This function is integrated over all three-space, with the ϕ and β defined in Figure 2. Note that in Figure 2 the x, y coordinates are in the plane of the hide.

Because of the very thin sections used in this study (60 μm), it is reasonable to consider the fiber orientation to be independent of β. The use of thin sections is warranted here; only the fiber orientation with respect to a plane perpendicular to the plane of the hide (i.e., parallel to the z-axis of Fig. 2) is of interest. Hence, equation (1) reduces to:

$$\langle \cos^2 \phi \rangle = \frac{\int_0^{\pi/2} I(\phi) \sin \phi \cos^2 \phi \, d\phi}{\int_0^{\pi/2} I(\phi) \sin \phi \, d\phi b} \tag{2}$$

Here, $I(\phi)$ represents the scattered light intensity (measured by the photocell) vs. ϕ, measured at a particular scanning radius r, defined in Figure 3. Figure 3 also shows the coordinate system of the SALS pattern itself, with the meridian parallel to the z-axis of the sample and the equator lying in the x-y plane. Only the orientation of the sample with respect to the z-axis is important in performing a SALS measurement, since fiber orientation is assumed to be independent of β.

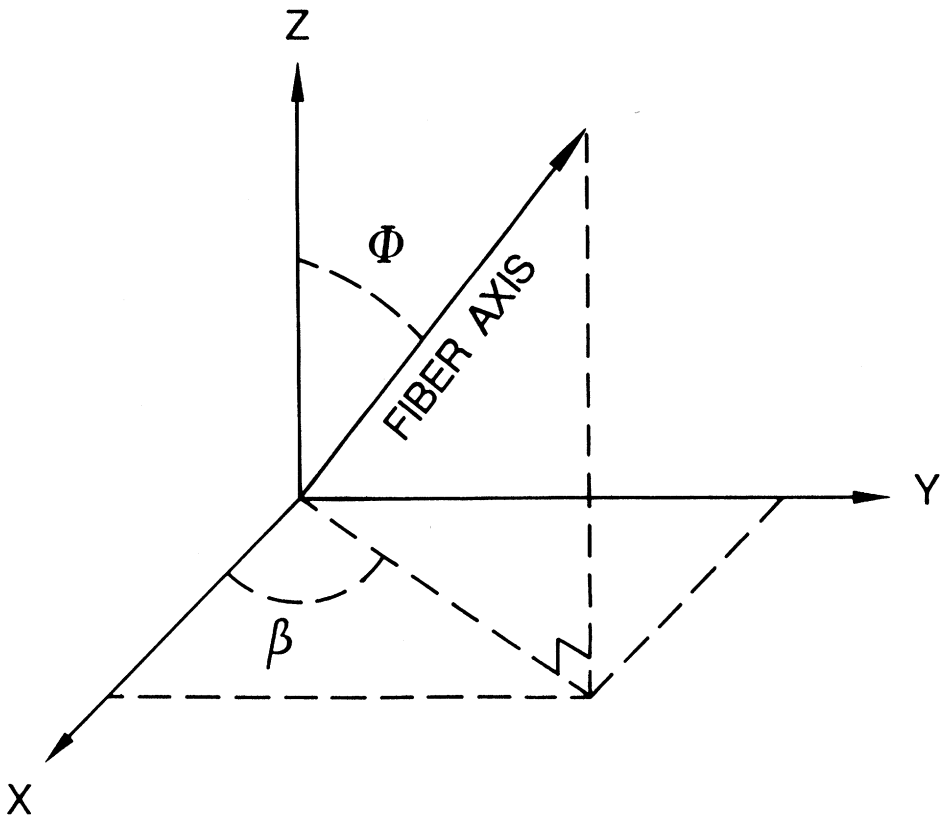

FIGURE 2 Coordinate system used to analyze fiber orientation in hide. *x-y* plane is in the plane of the hide.

Angles ϕ' of the scattered light rays are measured from the equator, because vertical fibers scatter light horizontally. For a given fiber, $\phi = \phi'$.

The mean-square cosine $\langle \cos^2 \phi \rangle$ is a single value that represents the degree of orientation in the sample with respect to a chosen axis, chosen here as the meridian. To express the values of $\langle \cos^2 \phi \rangle$ in a more convenient form, the following equation will be used.

$$OI = \tfrac{1}{2}[(3 \langle \cos^2 \phi \rangle - 1)]100\% \tag{3}$$

OI is an orientation index, used so that values for $\langle \cos^2 \phi \rangle$ can be transformed to a percent scale. A value of 100% corresponds to a sample perfectly oriented along its *z*-axis, 0% for a randomly oriented sample, and −50% for a sample perfectly oriented in the *x-y* plane (i.e., the plane of the hide).

The methodology used here determines a net or effective orientation of the fibers within the diameter of the laser beam. Any type of looping or other irregularities reported[1,10] would contribute to the value for the $\langle \cos^2 \phi \rangle$. Therefore, the SALS technique includes all structural information of the collagen fibers, and the results represent the total orientation behavior of the fibers.

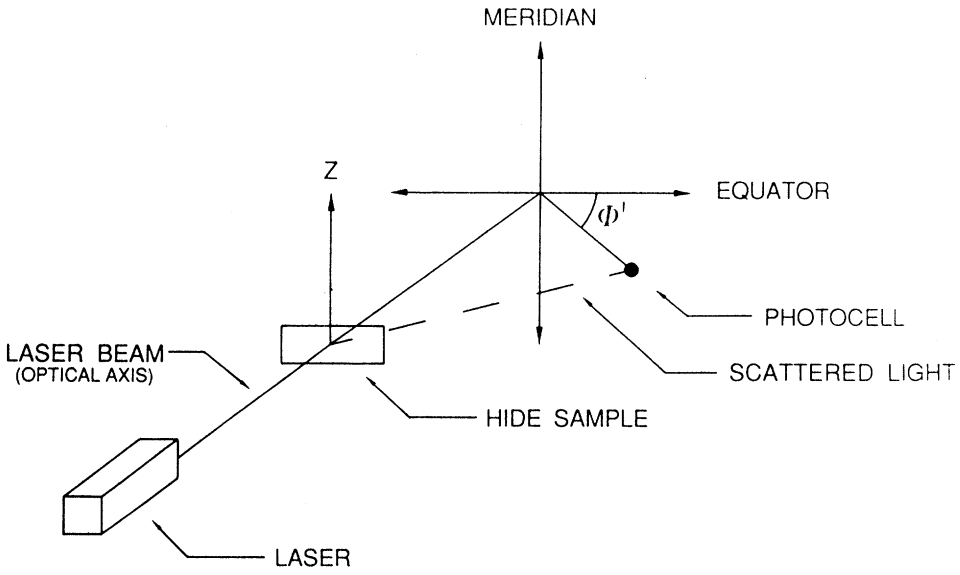

FIGURE 3 Orientation of a thin scattering sample with respect to the laser beam and photocell of the SALS optical system.

RESULTS

Preliminary experiments were first run to determine the optimal scan radius of the photocell. Larger structures within each sample (e.g., local irregularities) and histological artifacts such as tears tend to scatter close to the optic axis. The smaller collagen fibers, which are of interest, scatter light farther out from the optic axis. These scattering patterns are overlaid into a composite. To determine an optimal scan radius at which scattered light from the collagen fibers would dominate, entire scattering patterns of several sections were examined. Complete azimuthal scans (i.e., over the complete 360° range of ϕ) were taken at 2-mm intervals of radius r (Fig. 3), starting at the optic axis, outward to a maximum of 24 mm. It was found that the scan at the largest radius (24 mm) was the most sensitive to the orientation of the collagen fibers.

Figure 4 shows a surface plot of the scattered light intensity from a typical hide sample. Here the general nature of the scattering about the optic axis can be seen, especially the rapid decay in intensity as the scanning radius r increases. The rate of decay is dependent on direction, due to the oriented fibrous structure of the sample. Those samples with a preferred fiber orientation along their z-axes (meridional orientation) will tend to scatter more light in the equatorial direction. Thus the rate of decay of scattered light intensity will be less along the equator than along the meridian. The reverse is true for samples with a preferred orientation in the equatorial direction (in the plane of the hide).

Figure 5 shows two contour plots of the entire patterns of light scattered by a vertical-type VFD sample. In both plots the contours are plotted at the same intervals to facilitate comparisons. In the pattern from the upper reticular dermis (Fig. 5A) elliptical contours can be seen, with the long axis of the ellipses parallel to the equator. This indicates that the fibers

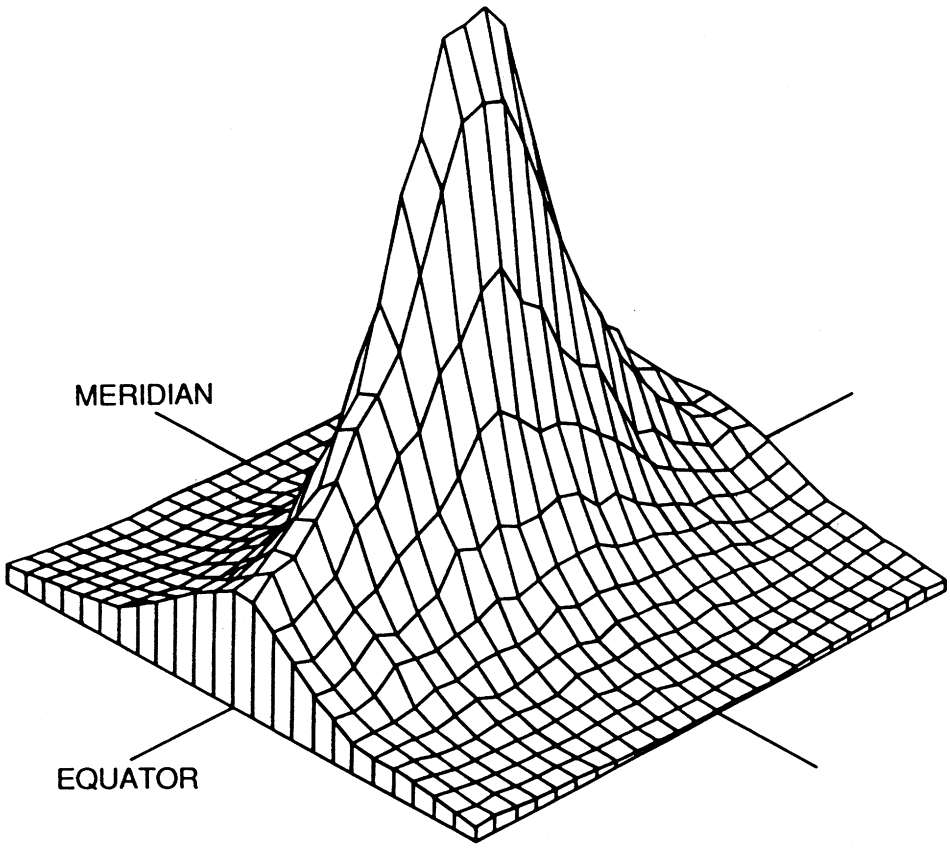

FIGURE 4 Distribution of light intensity in a typical scattering pattern from a vertical-fiber sample in the optical system of Fig. 3. The peak is centered on the laser beam.

have a preferred orientation along the meridian (i.e., vertical to the plane of the hide). The elliptical contours are expected because the scattering pattern consists of two streaks on the equator extending from a spot of maximum intensity at the origin. A preferred orientation of fibers in any one direction would give a family of concentric ellipse-like contours with their major axes perpendicular to that direction. The more circular contours of Figure 5B are the result from the same sample as that of Figure 5A, but taken from the lower reticular dermis. The circularity of these contours, corresponding to scattering along the meridian of similar intensity to that along the equator, indicates that there is a greater proportion of horizontal fibers and fibers oriented at 45° than in the upper layer represented by Figure 5A. Actually, on quantitative analysis of the data, a slight tendency for the contours to be extended along the meridian was found, indicating a slightly greater proportion of fibers oriented in the plane of the hide in the lower reticular dermis than in other directions. These plots agreed with histological findings for a VFD sample reported by Peters and Bavinton,[10] who also reported the fibers to be oriented vertically in the upper reticular dermis, but in the plane of the hide in the lower reticular dermis.

The other selected samples were examined with the SALS device at the maximum scan

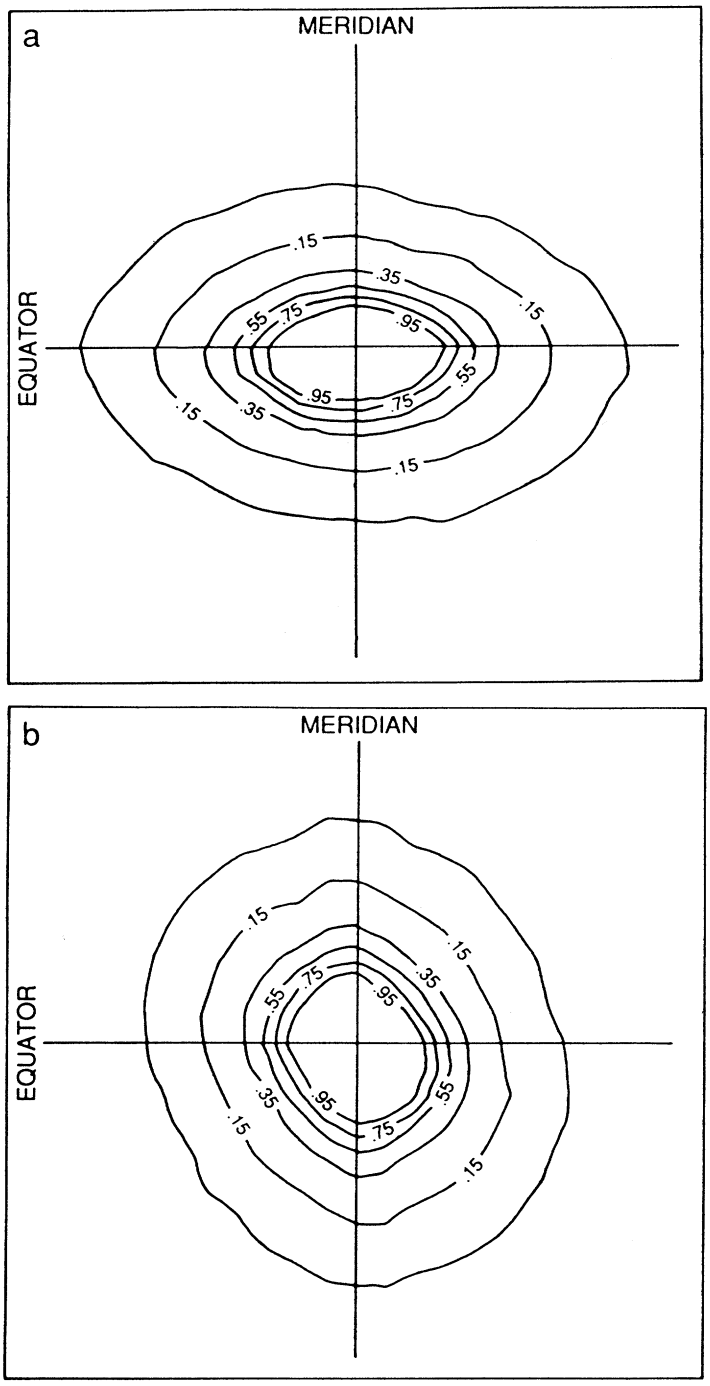

FIGURE 5 Contour plots of the intensity of light scattered from a vertical-fiber sample, from A, upper reticular dermis and B, lower reticular dermis. The contours represent arbitrary relative levels of light intensity, the same for the two plots. The orientations of the plots are the same as in Fig. 3.

radius of 24 mm. Values of the *OI* for each phenotype and dermal location are plotted in Figure 6. Here, variation in fiber orientation with dermal level and between normal and affected animals is clearly seen. The fibers in the upper dermis of the normal animals are randomly oriented (*OI* value near 0). Those similarly located in the intermediate-type and vertical-type upper dermis are vertical (larger *OI* value). Further, it is clear that the tissue with the preferred vertical orientation of fibers is found only in the upper layers of the dermis; fibers in the lower layer on the average lie parallel to the surface, giving negative *OI* values. Thus, VFD is observable only in upper dermis, regardless of phenotype. This important finding also indicates that the "high-weave" phenotype described by Peters and Bavinton[10] is derived from a visual impression from features, other than the preferred orientation of the fibers, that SALS fails here to distinguish.

The results of an analysis of variance of the data, blocked into a nested, partially hierarchical model, are shown in Table II. The column of F's gives the ratios of the variations of *OI*'s from particular sources of variability to the residual variation in the population ("Error"). F for a given source depends on the number of "degrees of freedom" (DF), which reflects the number of experimental observations, adjusted to the nature of the sources of

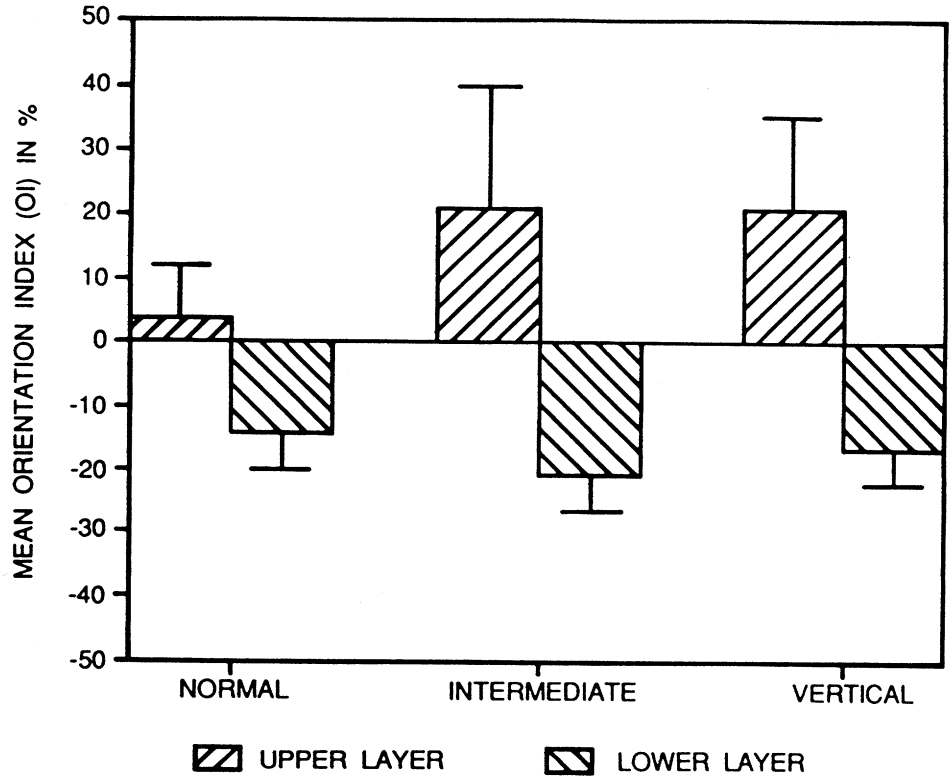

FIGURE 6 Orientation indices of fibers in upper and lower reticular dermis from the three phenotypes previously classified N, I, or V. The bars represent standard error of the mean. The upper layers of the vertical and intermediate hides have significantly greater vertical orientation than those of normal hide but do not differ significantly from each other. Fibers in the upper layers are always more vertical than those in the lower layers.

TABLE II

Anova tables of phenotypes, individuals within phenotypes, layers of dermis, phenotype-layer interactions, and microtome sections within individuals using a nested model

Source of Variability	DF	Mean Square	F	Probability > F
Phenotypes	2	629.15	4.55	.0125
Individuals within a phenotype	9	187.21	1.35	.2166
Layer in dermis	1	38638.45	279.64	.0001
Phenotype-layer interaction	2	1927.64	13.95	.0001
Section	12	59.86	.43	.9472
Error	117	138.17		

variation.[17] The last column is the probability that such a ratio (or larger) could come from the same random population. The first line shows that the three phenotypes belong to different subpopulations, with a confidence level well above 95% ($p < .0002$). The locus of the vertical fibers in the upper dermis appears as a strong interaction between phenotypes and level of dermis. That is, with a probability well above the 95% confidence level ($p < .0001$), the variation in *OI* with phenotype depends on which dermal layer it is from. While the variation between the two layers is shown to be highly significant ($p < .0001$), that from one section to another, in the same layer and individual, is not ($p < .9472$).

DISCUSSION

The visual impression derived from histological examination of VFD hides is that there is variation in the degree of expression of the trait. This can be due to the complicated texture of bovine hide, which confuses the eye—features such as nearby hair shafts, intruding fibers, the remnants of fibroblastic cells, and thick, prominent fiber bundles. The image can be "cleaned up" by optical filtering in the back focal plane of the microscope, but we have chosen to use the light pattern in this plane, the SALS, to obtain quantitative data on the fiber orientation.

There has been some controversy about the degree of expression of the VFD trait. While Cundiff *et al.*[2] show that a major gene is most likely to be involved, the existence of animals with an intermediate degree of "verticality" raised serious questions about how controlling such a gene could be. Here, by analyzing the statistical variance (ANOVA) in the *OI*'s determined from an objective physical measurement instead of comparing subjective impressions, we have shown that the upper stratum of reticular dermis from animals classified genetically and phenotypically as vertical or intermediate are quantitatively different from normal specimens. The analysis is consistent with Figure 6.

VFD was found to occur in only one form. The data for the upper layer (Fig. 6) does not distinguish the intermediate type from the vertical type. Moreover, a Bonferroni *t*-test,[17] which groups mean values according to significance, shows that the minimum significant difference ($p < .05$) between two values of *OI* in the upper layer is 10.7; therefore, the

vertical type is statistically indistinguishable from the intermediate type, but is different from the normal. Although there is variation among phenotypes for the lower dermis also, Figure 6 shows this variation to be very similar to that among individuals. The Bonferroni *t*-test applied to the lower layer shows that differences in means from normal and vertical types are insignificant ($p < .05$), the minimum significant difference in *OI* being 3.5. All lower layers have fibers parallel to the plane of the hide, but the fibers are significantly more so in the intermediate phenotype. We conclude that the concept of the intermediate phenotype is based on subjective histological observations only, with no measurable structural difference found between it and the vertical phenotype.

No evidence from fiber-orientation data was found for the occurrence of a "high-weave" phenotype.[10] The structural transition from upper to lower layers is sharp and well defined in all phenotypes, particularly so in those with VFD, as shown by the phenotype-layer interaction in Table II. A high-weave sample would have shown the fibers to have preferential vertical orientation throughout the dermis. For the Hereford samples used in this study, this was definitely not the case. In fact, the lower layer is almost completely unaffected by the occurrence of VFD in the upper dermis, with the possible exception of a significantly higher degree of orientation in the lower layer of intermediate type than in that of the normal and vertical phenotypes.

SALS has been used previously to examine textile fibers,[18] wood,[19] and leather.[16] By comparing the orientation functions with those determined from the arcs in the wide-angle X-ray diagram obtained on full-thickness samples of the same preparations, the workers of reference[16] demonstrated the validity of the techniques that we have used here. The automated SALS device used here has clearly shown its ability to precisely quantify the collagen fiber orientation in fixed dermal sections. With the use of *OI*, the fiber orientation has been characterized with one simple constant. The basic properties of bovine hide fiber orientation, in both the normal and pathological states, were easily distinguished. Even though OI's from both upper layers, where verticality is strong, and lower layers, where it is weak, were averaged together, the variability with phenotype is significant above the 95% confidence level ($p < .0125$). This would have been true even if dermal layer segregation had been ignored and the whole dermis had been sampled with the laser, using only six replications. A reliable value of OI for the vertical-fiber areas, however, cannot be determined in this way. Thus the mean values over all values of OI taken within each phenotype would be only -5.1% ($\pm 6.0\%$), 0.1% ($\pm 12.9\%$), and 1.9 ($\pm 11.1\%$), for normal-type, intermediate-type, and vertical-type, respectively. The strong variation of fiber orientation with level in the dermis is still confirmed ($p < .0001$). Disparities in subjective histological observations have also been clarified. Future work should include characterization of the collagen fiber orientation both throughout the hide and among breeds. Of special interest is the study of the structural transition from the upper and lower layers in phenotypes with VFD.

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